THE PATTERNING OF GLUTARALDEHYDE-CROSSLINKED GELATIN

[1]*Wei-Chih Lin and* [2]*Lung-Jieh Yang*

[1]Electronic Research & Service Organization (ERSO), Industrial Technology Research Institute (ITRI), Hsin-Chu, Taiwan ROC

[2]Department of Mechanical & Electro-Mechanical Engineering, Tamkang University, Tamsui, Taiwan ROC

ABSTRACT

This paper proposes a novel technique for fabricating micro patterns of glutaraldehyde(GA)-crosslinked gelatin. It provides another means to crosslink gelatin other than using the photo-sensitizing agents, and the micro patterns of GA-crosslinked gelatin can still be made successfully by accessing the conventional photolithography. The much less toxic and more biocompatible approaches of strengthening the gelatin microstructures can be developed according to the idea herein. The over-crosslinking or the edge-diffusion phenomena, and the correlated processing issues are also depicted in this paper.

1. INTRODUCTION

Gelatin, polymer from natural sources, is a biodegradable, biocompatible material, and first proposed to be used as a protection layer for low-temperature surface micromachining in MEMS'02 [1-2]. The natural gelatin's drawback of good dissolving in aqueous environment requires the crosslinking procedure by some appropriate agents. After being added with some photo-sensitizers, e.g., potassium dichromate ($K_2Cr_2O_7$), the gelatin gel acts like a negative-toned photoresist, and can be used to fabricate many micro patterns with the properties of good mechanical strength and good resistance to chemicals. However, the photo-sensitizers are always highly toxic and forbidden the practical application in the biomedical environment. It pushed many biomaterial researchers turn to use other kinds of crosslinking agents, such as formaldehyde [3], glutaraldehyde (GA) [4], carbodiimide [5] and dextran dialdehyde [6]. The gelatin strengthened by these functional group agents really has the more superior characteristics in biocompatibility, mechanical strength, anti-water transmission and anti-swelling. However, the method of fabricating GA-crosslinked patterns with micrometer size has not yet been reported in MEMS field.

2. FABRICATION METHOD AND PRELIMINARY RESULTS

In this paper, the authors combined the concepts of GA-crosslinking and the conventional photolithography to fabricate the gelatin microstructures. The fabrication process is depicted in Fig. 1. First, we spin-coats a gelatin film on a glass substrate [7]. Second, a masking layer of positive-toned photoresist (e.g. AZ-4620) is spun on the gelatin surface, and the correlated ultra-violet (UV) exposure for the portion of photoresist which defines the crosslinked gelatin is done. All the processing temperature of the above photolithography must be well controlled near the ambient temperature to prevent the melting of the natural gelatin underneath the photoresist.

Third, we dip the sample in GA solution, e.g., 25% in weight, to undertake the crosslinking reaction with proper control of time. Finally the GA-crosslinked micro patterns show up after both stripping the masking layer of photoresist by acetone and removing the uncrosslinked gelatin in hot water, sequentially. One example of the completed micro patterns is shown in Fig. 2. The finest line width that can be achieved in Fig. 2 is 40 μm. The thickness of the GA-crosslinked gelatin, depends on the ultimate spin-coating characteristic of the gelatin gel, can be made up to 10 μm. Fig. 3, which is measured by *alpha-step-500*, shows the surface profile of the 10 μm thick GA-crosslinked gelatin.

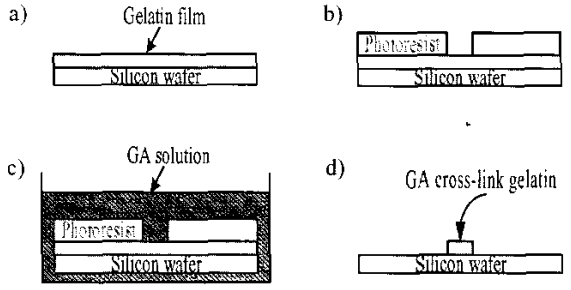

Figure 1: The fabrication process of GA-crosslinked gelatin: (a) spin-coating gelatin film; (b) photolithography; (c) dipping in GA; (d) stripping photoresist and pure gelatin.

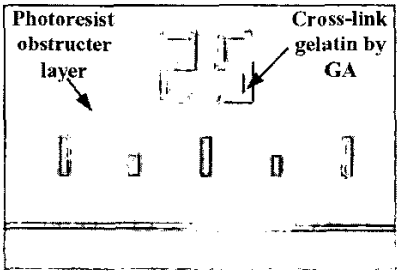

Figure 2: The GA-crosslinked gelatin patterns with the proper control of crosslinking time.

0-7803-8265-X/04/$17.00 ©2004 IEEE.

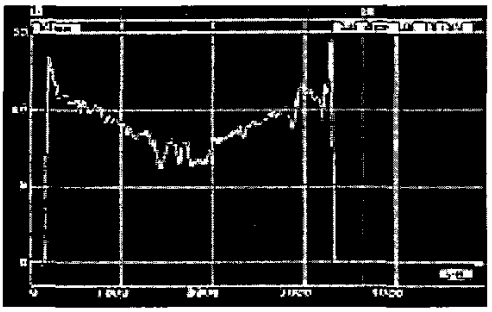

Figure 3: The surface profile of the GA-crosslinked gelatin pattern with the thickness up to 10 μm. The corrugated surface denotes the attack from alkaline developer during the photolithography of masking resist.

The corrugated surface profile of the crosslinked gelatin in Fig. 3 shows the poor surface morphology after the photolithography of masking resist and the GA crosslinking process. We explain this result due to the reason of the weak resistance of pure gelatin vulnerable to the high pH-valued alkaline developer (AZ400K) at the end of the resist development. In other words, no sooner had the resist-developing process ended, than the pure gelatin exposed to the alkaline developer and was etched immediately. Fig. 4 depicted the view of pure gelatin surface attacked by AZ400K developer.

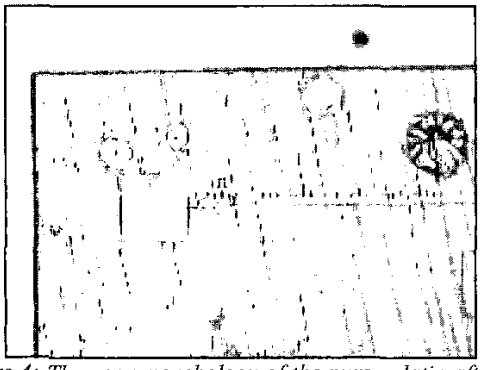

Figure 4: The poor morphology of the pure gelatin after the resist-development in AZ400K. The swelling strips due to the water-absorption of gelatin, and the volcano-shaped cracks all over the pure gelatin film reveal the uncertainty during the fabrication process.

The protection way against the attack of alkaline developer herein is to under-develop the masking resist. In other words, we decrease the developing time and recess thin layer of the UV-exposed photoresist on purpose. The residual layer of the UV-exposed photoresist, with several tens nanometers thick only, can provent the underneath pure gelatin from the attack of the developer, and can be removed rather easily by O_2 plasma (just as so-called "de-scum" process) before GA crosslinking process.

3. THE ABNORMAL PROFILE WITH TOO MUCH TIME OF GA-CROSSLINKING

After both stripping the masking layer of photoresist by acetone and removing the uncrosslinked gelatin in hot water (50°C), the GA-crosslinked micro patterns are supposed to appear. However, some residual of the gelatin near the edge of micro patterns still left on the substrate. Once again, the O_2 plasma "de-scum" process (with 100W for 1-2 min., too much time inducing cracks on gelatin) is mandatory and required after the hot water developing. The micro patterns with high-contrast and without fringe hue will show up accordingly.

Due to the intrinsic diffusion mechanism of GA molecules inside the gelatin chain-like protein, or due to the finite adhesion strength between the gelatin surface and the masking photoresist, the over-crosslinking at the edge of gelatin happens fairly often. The abnormal and imperfect gelatin patterns extrude from the edges by the observation of the "cross" and the "number" patterns in Figs. 5(a) and 5(b). We thereafter measured the surface profile across a gelatin line which has the over-crosslinking edges. As shown in Fig. 6, the original line pattern is confined by the trail with twin-peak, B and C.

Figure 7 describes the quantitative data of the lateral over-crosslinked distance vs. crosslinking time of gelatin subject to different weight percentage concentrations of GA solution. The square-rule behavior of the gelatin edge front with respect to the crosslinking time in Fig. 7 demonstrates that the respective plateau areas in A-B and C-D in Fig. 6, extruding laterally from both sides of the original line-pattern, is mostly controlled by the diffusion mechanism of GA agent in pure gelatin.

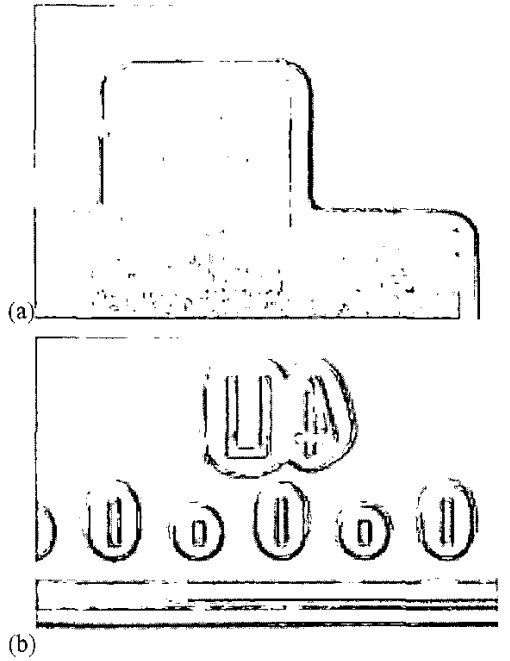

Figure 5: The GA-crosslinked gelatin patterns with over-time control of crosslinking: (a) a partial view of a "cross" pattern with the arm width of 1000 μm; (b) the straight line and "number" patterns with the line-width of 100 μm.

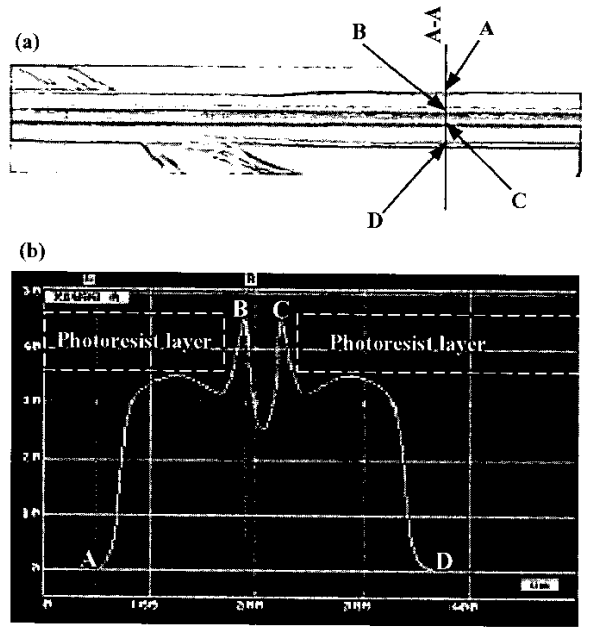

Figure 6: The surface profile of a GA-crosslinked gelatin pattern with over-time control of crosslinking: (a) the location definition of the measuring points; (b) the profile measured by Alpha-Step-500.

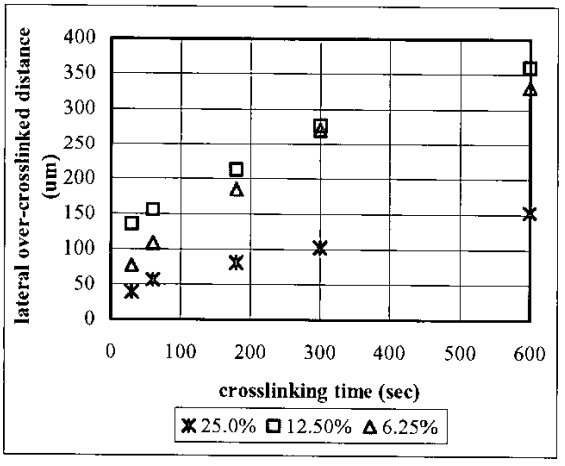

Figure 7: The lateral over-crosslinked distance versus crosslinking time of gelatin subject to different weight percentage concentrations of GA solution.

From Fig. 7, we also observe that the crosslinking time of GA solution for gelatin should be less than 1 min for avoiding large dimension error at the edge of micro patterns. Moreover, the high weight percentage concentration of GA solution, e.g., 45 %, is highly recommended for using because the less amount of dissolved water in GA solution induces less serious problem of undesirable gelatin swelling. Additionally, the high concentration of GA solution is also beneficial to the fast crosslinking of gelatin for preventing the overgrowth issue at the gelatin edge as just mentioned before.

4. THE MODIFIED PROCESSING AND RESULTS

Combining the processing remedy dealing with the undesirable issues described in the previous sections, we summarize a modified process of GA crosslinking for gelatin micro patterns:

(1) Spin-coating pure gelatin film at 40°C.

(2) Photolithography of a positive-toned resist with under-development at room temperature.

(3) O_2 plasma de-scum (100W, 1-3 min.) for the masking photoresist.

(4) Crosslinking in 45% GA solution with proper time control, e.g., 1 min.

(5) Stripping photoresist by acetone and removing pure (uncrosslinked) gelatin by hot water (50°C).

(6) O_2 plasma de-scum (100W, 1-2 min.) for the residual pure gelatin.

By the above modified process, some micro patterns with better morphology and contrast are fabricated and shown in Figs. 8 and 9. The thickness of the gelatin patterns in Figs. 8 and 9 is about 1.5 μm. The reliable minimum line-width is as good as 5 μm, and the minimum gap between two patterns is 10 μm so far.

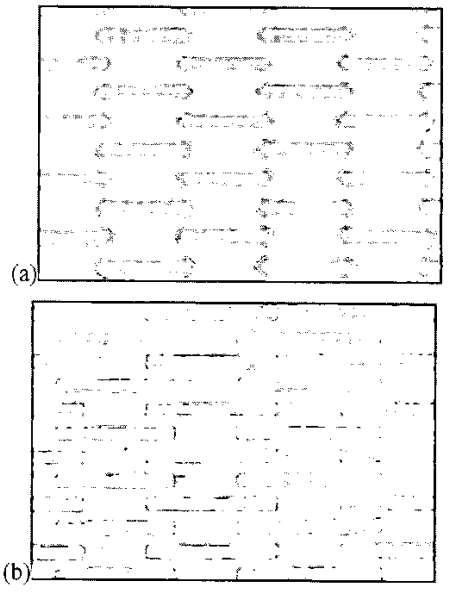

Figure 8: The gelatin patterns of 1.5μm thick microstrips lines by the modified GA-crosslinking process: (a) 30 μm long, 5 μm wide and 5 μm gap; (b) 100 μm long, 5 μm wide and 5 μm gap.

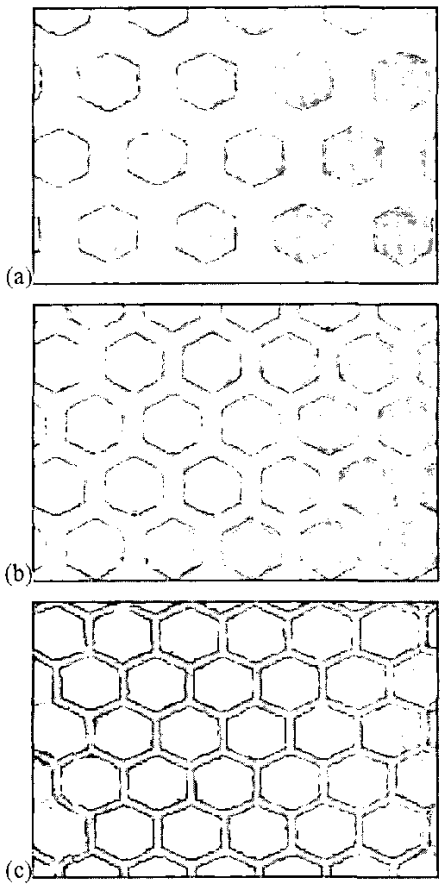

Figure 9: The micro honeycomb-shaped gelatin patterns with an edge of 50μm by the modified GA-crosslinking process: (a) 63 μm gap; (b) 33 μm gap; (c) 13 μm gap.

5. CONCLUSIONS

In summary, the patterning technique and the fabricated samples of GA-crosslinked gelatin are initially studied in this paper. We addressed and explained the over-crosslinking or the edge-diffusion phenomena of GA solution in gelatin accordingly. The modified processing procedure for GA-crosslinking gelatin is also proposed and realized herein. We hope to extend this methodology to other far less toxic crosslinking agents, e.g., the natural occurring agent "genipin" for crosslinking gelatin micro patterns in the very near future [8]. We believe that the biocompatibility, mechanical strength, chemical resistance, anti-water transmission and anti-swelling of the well cross-linked gelatin will provide useful microstructures to many applications in MEMS or even NEMS fields.

ACKNOWLEDGEMENTS

The authors want to appreciate the financial support of National Science Council of Republic of China (Taiwan ROC) with the research project number of NSC-91-2218-E-032-002. The process facilities provided by Micro System Laboratory (MSL) of ERSO, ITRI, and from Instrument & Experiment Center (IEC) of Tamkang University, are also highly acknowledged for using in this work.

REFERENCES

[1]L.-J. Yang et al., "Photo-patternable gelatin as protection layers in surface micromachinings", *Proceedings of the 15th IEEE MEMS conference*, Las Vegas, Jan. 20-24, 2002, pp. 471-474.

[2]L.-J. Yang et al., "Photo-patternable gelatin as protection layers in low-temperature surface micromachinings", *Sensors and Actuators A: physical,* vol. 103, no. 1-2, pp. 284-290, 2003.

[3]R. Niekisch, *Dtsch. Zahn Mund Kieferheik. Zentralal.*, vol. 67, p. 21, 1979.

[4]P.M. Neumann et al., *Journal of Biomedical Materials Research*, vol. 15, p. 9, 1981.

[5]Y.S. Choi et al.,"Studies on gelatin-containing artificial skin:II. Preparation and characterization of cross-linked gelatin-hyaluronate sponge", *Journal of Biomedical Materials Research*, vol. 48, no.5, pp. 631-639, 1999.

[6]J.P. Draye et al., " In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films", *Biomaterials*, vol.19, no.18, pp. 1677-1687, 1998.

[7]*Preparation of pure gelatin film on a glass substrate:* Dissolve gelatin powder in water at 40-50°C. Filter to remove bubbles. Apply to glass plates by spin-coating at 40°C. Dry at room temperature for 3-4 hours.

[8]W.-H. Chang et al., "A genipin-crosslinked gelatin membrane as wound-dressing material: in vitro and in vivo studies", *J. Biomaterial Science Polymer Edition*, vol. 14, no. 5, pp. 481-495, 2003.